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# THE MATURATION OF *HYDROIDES DIANTHUS*[1]

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The most predominate technique used in marine fouling research is the evaluation of biological growths on immersion panels or test rafts exposed to the ocean environment (DePalma, 1964). A new approach to this problem is the development of a laboratory bio-assay procedure. A laboratory procedure in fouling research offers a technique which can provide reliable quantitative results rapidly under controlled conditions. An assay type test for marine fouling studies was first suggested by deSilva (1958) in his studies of the settling characteristics of the polychaete, *Spirorbis*. With *Spirorbis* as the assay organism, Meadows and Williams (1963) obtained results on substrate selectivity in twelve hour tests. Wisely (1963a, 1963b) and Freiberger and Cologer (1965) have also reported on the use of bio-assay techniques in marine fouling studies. In this regard the marine polychaete tubeworm, *Hydroides* (*Eupomatus*) *dianthus*, (Verrill, 1873), a common fouling organism, is a potential assay organism. A bio-assay procedure implies the mass rearing of the animal in the laboratory, the maintenance of mature adults, and the conditioning and maturation of adults for out of season spawning. Although some marine invertebrates have been well studied in the laboratory, (Loosanoff and Davis, 1950; 1951; 1963; Loosanoff, 1954; Moyse, 1960); little has been published concerning the mass rearing and maturation of the worm *H. dianthus* in the laboratory (Turner and Hanks, 1960; Wisely, 1958). The latter paper is concerned with *H. norvegica*, a closely related species.

The objective of this project was to examine the general problems pertinent to the maintenance of laboratory populations of *H. dianthus*, to maintain and culture suitable marine micro-algae as food types for worms, and to conduct multi-factorial experiments in determining the effects of temperature, food type, food concentration, salinity, and artificial sea water, on the maturation of the worm in the laboratory.

## Materials and Methods

### *Algal food*

Four marine algal species were examined as food for the tubeworm; *Phaeodactylum tricornutum* (Bohlin), *Isochrysis galbana* (Parke), *Dicrateria inornata*, and *Nannochloris* sp. Cultures were obtained from Dr. R. Guillard, Woods Hole

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Oceanographic Institution, Woods Hole, Massachusetts, and maintained in a growth medium recommended by Dr. Guillard, but modified slightly to include the following: (1) commercially prepared artificial sea water, (Rila Marine Mix, Teaneck, New Jersey; Instant Ocean, Aquarium Systems, Inc., Wickliffe, Ohio), (adjusted to 30‰ salinity) in lieu of natural sea water; (2) tris (hydroxy-methyl) amino methane buffer (1 g/l), and (3) a higher concentration of nitrate (204 mg/l as N).

Techniques used for the mass culture of algae were similar to those reported by Loosanoff and Davis (1963) and Ukeles (1965).

*Maturation experiments*

The maturation of worms in the artificial environment provided in the laboratory necessitates the study of the effects of some important environmental parameters on the worm. Some of these factors include temperature, food type, food concentration, salinity, and water type. Two multi-factorial experiments were planned in order to examine all the variables.

The first experiment (Experiment I) consisted of three separate factorial experiments. These involved three food types: *Phacodactylum tricornutum*, *Isochrysis galbana*, and *Nannochloris* sp. Each was evaluated at three levels of supply. Two runs were made with each food type; one with artificial sea water (Rila Marine Mix), the other with natural sea water. Artificial sea water was prepared according to instructions supplied by the manufacturer. Natural sea water was obtained at Noank, Connecticut and was passed through a 0.45 micron membrane filter to remove natural food. Salinity was maintained at 30‰. At times it was necessary to increase the natural salinity of the sea water by heating and evaporation in order to achieve 30‰ salinity.

Temperature was maintained at three levels: 10° C, 16° C, and 22° C by temperature controlled aquaria. Each test beaker was aerated. The experimental variables tested in Experiment I are shown in Table I. Note that fifty-four tests were conducted and included two variables at three levels, two types of sea water, and three food types. The separate beakers were assigned levels of temperature, food concentration, and food type randomly.

Adult worms were maintained in a low-temperature aquaria (5° C) in natural sea water (30‰) for at least two weeks. The intent was to arrest or prevent gametogenesis. The sea water was continuously circulated through an ultraviolet irradiation chamber to keep bacterial populations to a minimum. No food was supplied to the worms during this period.

Prior to initiating an experimental run, four worms were taken from the preconditioning tank and removed from their tubes and examined for ova or sperm. In all cases the gametes were found to be immature or undeveloped.

A number of worms of approximately equal size were placed into two-liter beakers containing one liter of the appropriate medium (natural or artificial sea water) and the prescribed concentration of food added. Worms to be used at the higher temperatures were conditioned gradually to increasing temperatures at two day intervals until the desired temperatures were reached. At this point the factorial experiment was initiated. The sea water was changed and food pro-

TABLE I

*Experimental variables evaluated—Experiment I*

| | | Sea water type | | | | | |
|---|---|---|---|---|---|---|---|
| | | Artificial | | | Natural | | |
| | | Food concentration (mg [dry] algae/worm/2 days) | | | | | |
| | | $C_1$ (0.003) | $C_2$ (0.03) | $C_3$ (0.3) | $C_1$ (0.003) | $C_2$ (0.03) | $C_3$ (0.3) |
| Temperature (°C) | $T_1$ (10) | $T_1C_1$ | $T_1C_2$ | $T_1C_3$ | $T_1C_1$ | $T_1C_2$ | $T_1C_3$ |
| | $T_2$ (16) | $T_2C_1$ | $T_2C_2$ | $T_2C_3$ | $T_2C_1$ | $T_2C_2$ | $T_2C_3$ |
| | $T_3$ (22) | $T_3C_1$ | $T_3C_2$ | $T_3C_3$ | $T_3C_1$ | $T_3C_2$ | $T_3C_3$ |

(a) In the experiment, three different food types were used: Run No. 1—*Phaeodactylum tricornutum*, Run No. 2—*Nannochloris* sp., Run No. 3—*Isochrysis galbana*. (b) Food concentration in the case of Run No. 1 was 0.02, 0.2, and 2.2 (mg [dry] algae/worm/2 days).

vided on alternate days for the duration of the experiment. Several worms were removed from each beaker and examined to determine the state of gametogenesis at intervals of approximately 12, 30, and 25 days.

Gonadial products were obtained by either removing the worm from its tube or breaking the tube and exposing the abdomen. Sexual products are immediately discharged from abdominal pores. Male products were a whitish discharge and females a pink discharge, (Grave and Oliphant, 1930; Wisely, 1958).

The following descriptive terms were used to describe the results:

NR—No Response—indicates absence of gametes
IO—Immature Ova—indicates small undeveloped ova (diameter less than 50 microns)
IS—Immature Sperm—indicates non-motile sperm
MO—Mature Ova—indicates mature ova (diameter greater than 50 microns)
MS—Mature Sperm—indicates motile sperm
PS—Probably Spent—indicates no response animals which were mature in a previous interval

A second multi-factorial experiment (Experiment II) was planned in order to evaluate the effect of salinity, and increased temperature levels on the maturation of the worm. This experiment involved three levels of temperature (22° C, 26° C, and 30° C), and three levels of salinity (15, 25, and 30‰). Three temperature controlled aquaria were utilized for the experiment. The sea water was artificial sea water (Instant Ocean) prepared at the three levels. Two variables at three levels or a total of nine combinations were tested. Two factorial experiments were managed simultaneously. *P. tricornutum* was used as the food organism in one factorial experiment; an algal mixture (*P. tricornutum, I.*

*galbana,* and *D. inornata*) was used in the other. Food was supplied at a concentration of 0.5 mg (dry) algae/worm/2 days.

Prior to initiating Experiment II, ten worms were examined for the presence of mature ova or motile sperm. In all cases the gametes were either immature or undeveloped.

The procedure for start-up of Experiment II was similar to that already described for Experiment I, except that worms were removed from beakers and examined for gametes at intervals of approximately 19, 24, and 31 days.

In addition to the descriptive response of maturity noted in Experiment I, male products were examined for motility, and counted in a Neubauer counting chamber. A drop of 10% formalinized artificial sea water was added to the sperm preparation in order to facilitate counting. Two counts were made and averaged. Female products were counted in a Sedwick-Rafter plankton chamber, and were sized and measured by a calibrated ocular micrometer. All sexual products were collected in either two or three ml of artificial sea water.

## Results

### *Experiment I*

The results of the factorial experiments on conditioning of the worms fed *P. tricornutum, Nannochloris* sp. and *I. galbana* were summarized, grouped, and is presented in Table II. The per cent mature worms observed for each category was computed.

Gamete maturation was observed by the 12th day, but generally required 17 days or more. Some 30 per cent of the worms were mature by the 20th day and 37 per cent were mature by the 25th day.

Table II

*Summary—Maturation experiment—Experiment I. effect of sea water, time, food, type, and temperature on maturation of* Hydroides dianthus

| | Mature | Immature | N. R. | P. S. | Number Total mature / number | Mature worms (%) |
|---|---|---|---|---|---|---|
| Sea water | | | | | | |
| Artificial Sea water | 54 | 92 | 16 | 19 | 54/181 | 29.8 |
| Natural Sea water | 37 | 77 | 30 | 21 | 37/165 | 22.4 |
| Time (Days) | | | | | | |
| 12 | 17 | 78 | 25 | — | 17/120 | 14.2 |
| 20 | 39 | 61 | 14 | 17 | 39/131 | 29.8 |
| 25 | 35 | 30 | 7 | 23 | 35/95 | 36.9 |
| Food type | | | | | | |
| *Phaeodactylum* | 23 | 45 | 6 | 3 | 23/77 | 29.9 |
| *Nannochloris* | 24 | 81 | 17 | 15 | 24/137 | 17.5 |
| *Isochrysis* | 44 | 43 | 23 | 22 | 44/132 | 33.2 |
| Temperature (°C) | | | | | | |
| 10 | 8 | 76 | 17 | 9 | 8/110 | 7.3 |
| 16 | 22 | 65 | 17 | 6 | 22/110 | 20.0 |
| 22 | 61 | 28 | 12 | 25 | 61/126 | 48.4 |

At the highest temperature level tested in Experiment I (22° C), 48 per cent of the worms examined had mature gametes. At 16° C and 10° C, the percentage of worms that had matured was 20 per cent and 7 per cent, respectively.

Of the three algal food types examined, *Nannochloris* sp. was apparently the least effective. Both *I. galbana* and *P. tricornutum* were more effective in the maturation of the worm.

There was slightly better results in the gametogenesis of worms held in artificial sea water (30%) than in natural sea water (22%).

A summary of the data according to sex is shown in Table III. Of a total of 346 worms used in the experiment, 91 worms were mature (26%), and 255 worms were either immature or did not respond. From a total of 70 male worms observed, 29 were mature (41%), and from a total of 190 female worms observed 62 were mature (33%). Note that only 86 worms out of 346 did not respond or 75% of the worms responded in one way or another.

TABLE III

*Summary—Maturation Experiment I. Response of the worms according to sex*

| | Males | Females | Total |
|---|---|---|---|
| Mature | 29 | 62 | 91 |
| Immature | 41 | 128 | 169 |
| No response | — | — | 86 |
| Grand total | 70 | 190 | 346 |
| % Mature | 41 | 33 | 26 |

*Experiment II*

The results observed in the factorial Experiment II were summarized and the percent mature worms for each category was computed and is presented in Table IV.

At intervals of 19, 24, and 31 days, mature gametes were observed in 46, 62, and 64 per cent of the worms, respectively.

Low salinities affected worm maturation. Only 14% of the worms matured at 15‰ salinity, compared with 79% and 78% maturing at 25 and 35‰, respectively. Note that all deaths during the experiment occurred in those beakers having salinities of 15‰. In those worms surviving at 15‰ salinity, little or no tube growth was observed.

Maturation was not increased by subjecting worms to increasing temperature. Slightly more worms matured at the lower temperature (22° C) than did at the higher temperatures of 26° C and 30° C. Nevertheless, greater than 50% of the worms matured at all temperatures.

No difference in maturation was noted in feeding worms either *P. tricornutum* or an algal mixture. Worms matured fairly well fed either type of food. Table IV also presents maturation data based on the response of female and male worms. Generally, no significant changes occurred, except that increasing temperatures and a mixed food enhanced the maturation of females, but not males.

TABLE IV

*Summary—Maturation experiment—Experiment II. Effect of food type, salinity, temperature, and time on maturation of* Hydroides dianthus

| | Mat. | Im. | N. R. | Dead | No. mat. / Total number | Mature worm (%) | Mature males (%) | Mature females (%) |
|---|---|---|---|---|---|---|---|---|
| Food type | | | | | | | | |
| Mix | 42 | 9 | 14 | 11 | 42/76 | 55.2 | 45.3 | 78.4 |
| Phaeo | 50 | 16 | 16 | 12 | 50/89 | 56.1 | 53.8 | 59.4 |
| Salinity (‰) | | | | | | | | |
| 15 | 8 | 16 | 11 | 23 | 8/58 | 13.8 | 14.6 | 11.7 |
| 25 | 44 | 3 | 9 | — | 44/56 | 78.5 | 72.2 | 90.0 |
| 35 | 40 | 6 | 5 | — | 40/51 | 78.4 | 71.4 | 86.9 |
| Temperature (°C) | | | | | | | | |
| 22 | 33 | 15 | 9 | 1 | 33/58 | 57.0 | 63.3 | 50.0 |
| 26 | 27 | 7 | 13 | 1 | 27/48 | 56.2 | 41.4 | 78.9 |
| 30 | 32 | 3 | 3 | 21 | 32/59 | 54.3 | 45.7 | 84.5 |
| Time (Days ± 1) | | | | | | | | |
| 19 | 31 | 7 | 7 | 23 | 31/68 | 45.5 | 36.0 | 72.2 |
| 24 | 28 | 10 | 7 | — | 28/45 | 62.2 | 64.0 | 60.0 |
| 31 | 33 | 8 | 11 | — | 33/52 | 63.5 | 60.0 | 68.1 |

Code: Mat. = Mature worms; Im. = Immature worms; N.R. = No response.

A summary of the data according to sex is shown in Table V. Of a total of 138 worms used in this experiment, excluding dead animals, 92 (67%) were mature. Mature gametes were obtained from 52 male worms (93%) and 40 female worms (66%). Of 138 worms observed only 21 worms did not respond or 85% of the worms did respond in some manner.

TABLE V

*Summary—Maturation Experiment II. Response of worms according to sex*

| | Males | Females | Total |
|---|---|---|---|
| Mature | 52 | 40 | 92 |
| Immature | 4 | 21 | 25 |
| No response | — | — | 21 |
| Dead | — | — | 23 |
| Grand total | 56 | 61 | 161 |
| % Mature | 93 | 66 | 67* |

* Excludes dead animals.

*Fecundity of* Hydroides dianthus (*Experiment II*). The gametes released were collected and counted, either in a Neubauer counting chamber, or Sedgewick-Rafter plankton counting chamber. The values obtained in the case of males represent an average of two counts, however, in the case of females, all the ova in a one ml sample were counted. The data concerning gamete productivity were grouped, averaged, and summarized (Table VI).

Most sperm were obtained in those worms examined by the 19th day. Worms observed by the 24th and 31st day, resulted in decreasing numbers of motile

TABLE VI

*Summary—Effect of time, temperature, salinity, and food type, on number of mature gametes obtained in laboratory spawning of* Hydroides dianthus

| | Sex | No. worms | Average no. mature gametes/worm |
|---|---|---|---|
| Time (Days ± 1) | | | |
| 19 | M | 17 | 93,361,176 |
| | F | 8 | 12,208 |
| 24 | M | 16 | 65,870,187 |
| | F | 12 | 18,850 |
| 31 | M | 18 | 36,688,888 |
| | F | 15 | 49,975 |
| Temperature (°C) | | | |
| 22 | M | 19 | 92,150,000 |
| | F | 9 | 41,371 |
| 26 | M | 12 | 41,727,915 |
| | F | 15 | 37,006 |
| 30 | M | 20 | 52,493,750 |
| | F | 11 | 13,278 |
| Salinity (‰) | | | |
| 15 | M | 6 | 6,341,666 |
| | F | 2 | 13,162 |
| 25 | M | 25 | 78,462,920 |
| | F | 16 | 28,329 |
| 35 | M | 20 | 65,232,000 |
| | F | 17 | 34,935 |
| Food type | | | |
| Mix | M | 24 | 73,777,833 |
| | F | 16 | 27,580 |
| Phaeo | M | 27 | 49,288,703 |
| | F | 19 | 33,274 |

sperm present. The number of ova observed, however, increased with each increasing time interval.

Nearly twice as many sperm were obtained from worms matured at 22° C than at either 26° C or 30° C. The number of ova obtained in worms matured at 22° C and 26° C and were not significantly different, approximately 40,000 ova/worm; however, at 30° C only approximately 13,000 ova/worm were obtained.

The lowest salinity tested obviously affected gamete production. Small numbers of ova and sperm were attained at the 15‰ salinity. At the 25‰ salinity, some 78 million sperm/worm were obtained compared with 65 million sperm/worm at 35‰ salinity. With the mature ova, more ova were obtained at increasing salinities.

Food type has an effect on the number of sperm released. More sperm were obtained in those worms fed an algal mixture, than from those worms fed a single species. The ova did not appear to be grossly affected by the different food types, although slightly higher values were recorded in those worms fed only the diatom.

The male releases about two orders of magnitude greater numbers of sperm than do females releasing ova. The average male released some 62 million sperm while the average female released some 30 thousand mature ova.

## Discussion

The two multi-factorial experiments represented an effort to determine the influences of algal food type, temperature, salinity, water type, and conditioning time on the maturation of *H. dianthus* in the laboratory.

Artificial sea water prepared from commercially available synthetic sea water mixtures had little effect on maturation. Effective maturation occurred in the artificial sea water as well as in the natural sea water. The use of artificial sea water in the laboratory culture and maturation of the worm would be advantageous. It eliminates the problem of variability and uncertainty of natural sea water composition.

Mature gametes were observed in some instances in twelve days, however, a greater number of worms indicated maturity in twenty-five days. Increasing the conditioning time to thirty-one days did not yield more mature worms. In studies reported by Turner and Hanks (1960), maturation of *H. dianthus* was achieved between seven to ten days at 23° C in natural sea water.

All food types examined as food were effective in the maturation of the worm. *Nannochloris* sp. the smallest alga under study, was apparently the least effective. An algal mixture did appear to effect the maturation of female worms (Table IV).

Temperature affects gametogenesis. The per cent of mature worms was highest in those worms held at 22° C, and decreasing values were obtained with decreasing temperatures. Increasing temperature above 22° C did not enhance maturation of the male worms, highest values were attained with the 22° C level. However, female worm maturation was increased by increasing temperature (Table IV).

Low salinities affected worm survival and worm development. All deaths occurring in the experiment were observed at 15‰ salinity. The high temperature (30° C) and low salinity (15‰) accounted for 21 of the 23 deaths recorded. In a study with mollusc larvae, Davis and Calabrese (1964) reported that salinity affects temperature tolerance of these forms. Neff (1968), reports that *H. dianthus* is unable to produce mineral for tube building, when salinities are below 20‰. The low salinity level selected for evaluation was obviously unsatisfactory for the maturation and maintenance of the worm in the laboratory. The maturation of the worm was satisfactory in the range of 25–35‰.

An analysis of variance on the effect of temperature and salinity on male and female worms matured in Experiment II was completed. The effects of type of food and conditioning time were ignored for purposes of the analysis. The per cent mature worms were transformed by an arcsine transform to eliminate zero percentages. Female gamete maturation was significantly affected by both

temperature and salinity, at the 5% probability level. The interaction was non-significant. Temperature, salinity, and interaction effects were all non-significant in the case of male gamete maturation.

Of a total of 507 worms used in both experiments, 107 worms did not respond and only 23 deaths were recorded, or approximately 75% of the worms responded to the conditioning treatments. Remarkable response despite the use of wide ranges of temperature, food concentration, and salinities.

Mature gametes were observed in 41% of the males and 33% of the females in Experiment I, and in 93% of the males, and 66% of the females in Experiment II, only 16% of the worms were immature in Experiment II indicating that better conditions for maturation existed in Experiment II.

An estimate of the number of mature gametes released by *H. dianthus* is of interest. No information concerning gamete production was found in the literature. Tubeworm ova production was decreased by increasing temperature but increased by increasing salinity. Most mature ova were obtained in a conditioning time of 31 days, with decreasing values at 25 to 19 days, respectfully. The average number of mature ova released by a single worm was approximately 30 thousand. This represents a value averaged over all the conditions examined in the experiment. The number ranged from less than 100 to approximately 200 thousand ova/worm. Ova production in worms appears to be for less than that reported for molluscs. Davis and Chanley (1956), report that oysters produce some 50 million ova, and clams about 25 million ova.

The average number of motile sperm released by a single worm was 62 million. The number of sperm released ranged from 765,000 to greater than 400 million/worm.

In general, this study provides guide lines and ranges to permit the maintenance, culture, and maturation of the tubeworm *H. dianthus* in the laboratory. Optimum conditions were not achieved, yet, a high degree of maturation was accomplished in the laboratory environment.

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## Summary

1. All microalgae used as food were satisfactory food for the worm. (a.) *I. galbana* and *P. tricornutum* were better food organisms than was *Nannochloris* sp. (b.) An algal mixture of *I. galbana, D. inornata* and *P. tricornutum* proved to be slightly better than the single food species *P. tricornutum*. (c.) No effect of feeding level was observed. An algal food concentration of 0.25 mg (dry weight) algae/worm/day appears satisfactory.

2. Artificial sea water did not inhibit maturation.

3. Gametogenesis was directly related to temperature up to 22° C, the higher the temperature the greater the number of sexually mature worms. The number of mature worms was not increased appreciably above 22° C.

4. Worms were mature in 12 days of conditioning, however, more worms indicated maturity in 25 days.

5. Low salinity (15‰) affected worm survival and worm maturation.

6. A female worm expels an average of 30 thousand mature ova. The male worm releases about 62 million sperm. (a.) Sperm and ova production was decreased by high temperature (30° C), and low salinity; ova production increased with time but sperm production decreased with time. (b.) Fecundity was greater in worms fed an algal mixture.

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